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Isoindolo[2,1-*c*]benzo[1,2,4]triazines: A new ring system with antiproliferative activity

Patrizia Diana,* Annamaria Martorana, Paola Barraja, Antonino Lauria,
Alessandra Montalbano, Anna Maria Almerico, Gaetano Dattolo
and Girolamo Cirrincione

Dipartimento Farmacochimico Tossicologico e Biologico, Università degli Studi di Palermo, Via Archirafi 32, 90123 Palermo, Italy

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Abstract—A series of isoindolo-benzo-triazines of type **4** was obtained by diazotization of 2-(2-aminoaryl)-1-cyanoisoindoles **3a**–**j**. All the synthesized derivatives were screened by the National Cancer Institute (NCI, Bethesda, USA), for in vitro antitumor activity against a 3-human cancer cell line panel consisting of MCF7 (breast), NCI-H460 (lung), and SF-268 (CNS). Derivatives **4a**, **f**, **i**, **j** were selected to be evaluated in the full panel of about 50 human tumor cell lines derived from nine human cancer cell types and showed antiproliferative activity generally in the micromolar range. The most sensitive cell lines were: MOLT-4 and SR of the leukemia subpanel, A549/ATCC and EKVX of the nonsmall cell lung subpanel, COLO-205 of the colon cancer subpanel, LOX IMVI of the Melanoma subpanel, OVCAR-8 of the ovarian cancer subpanel, and MCF7, BT-549 of the breast cancer subpanel.

1. Introduction

DNA represents one of the most important cellular targets for several chemotherapeutic drugs. Polycyclic nitrogen heterocycles with a planar structure such as acridine, phenanthridine, and actinomycin derivatives can be good pharmacophores for classes of antitumor drugs since they can intercalate between the base pairs of double-strand DNA.[1] In connection with our studies on polycondensed nitrogen heterocycles, containing pyrrole or indole moieties with antineoplastic activity we reported the synthesis and the remarkable antiproliferative activity of indolo[3,2-*c*]cinnolines and indolo[1,2-*c*]triazines.[2,3] More recently we reported the synthesis of pyrrolo[2,1-*c*][1,2,4]triazines and [1,2,4]triazino[4,3-*a*]indoles that showed antitumor activity with GI_{50} values in the range 10^{-5}–10^{-6} M.[4] Several 1,2,4-triazine derivatives are well-known compounds endowed with a wide range of biological activities.[5,6] A large number of synthetic 1,2,4-triazines have shown antineoplastic activity in in vitro and in in vivo systems.[7–9] Benzocondensation to the 1,2,4-benzotriazines system led to compounds that showed IC_{50} 8–500 μg/ml for inhibition of growth of Hep-2, HeLa, peritoneal carcinoma, and pleurocarcinoma cells.[10] In particular, when condensed with one or more heterocycles, they found application in many fields such as pharmaceuticals, herbicides, pesticides, and dyes. For instance, pyrrolo[2,1-*f*][1,2,4]triazines exhibited activity against EGFR or VEGFR-2 kinase and many of them were shown to have substantial activity in cellular assays.[11,12] Also indazolylpyrrolotriazines showed inhibitory activity against tyrosine kinase of growth factor receptors such as HER1, HER2, and HER4, thereby making them useful as antiproliferative agents.[13]

2. Results and discussion

In our attempts to search for novel antitumor agents, we extended our interest to the isoindole system and planned to synthesize isoindolo[2,1-*c*]benzo[1,2,4]triazines **4a**–**j** with the aim of evaluating their antiproliferative activity.

2.1. Docking

Preliminary docking studies on the new series of the isoindolo[2,1-*c*]benzo[1,2,4]triazines were performed, using

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* Corresponding author. E-mail: diana@unipa.it

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AUTODOCK software, in order to evaluate by this fast in silico screening new potential DNA-interactive drugs. AutoDock combines a rapid energy evaluation through precalculated grids of affinity potentials with a variety of search algorithms to find appropriate binding positions for a ligand on a given receptor. Several runs of space search were carried out to find the best ligand–receptor binding arrangement. We performed automated docking studies on the isoindolo-triazines **4a–j** using Lamarckian Genetic Algorithm (LGA), which is the best search global algorithm.[14] As biomacromolecular target, we chose the DNA fragment identified by the Protein Data Bank (PDB) code 1DSD in which was originally intercalated the actinomycin D whose X-ray crystallographic structure has been downloaded from the PDB web site.[15] Before running autodock, it was necessary to prepare the DNA sequence: the original ligand and the water molecules were removed, obtaining a macro.pdb file, which contained only biomolecule coordinates.

The range of calculated free energy is from −19.96 to −24.47 kcal/mol and these values are lower then those calculated for Actinomycin D (−10.36 kcal/mol). The ligand with the highest affinity was the derivative **4j** which had −24.47 kcal/mol, and therefore it could be expected to form the more stable complex with DNA (Table 1). On the basis of these docking virtual screening results we supposed that isoindolo[2,1-*c*]benzo[1,2,4]triazines may be good candidates as new DNA-interactive compounds.

2.2. Chemistry

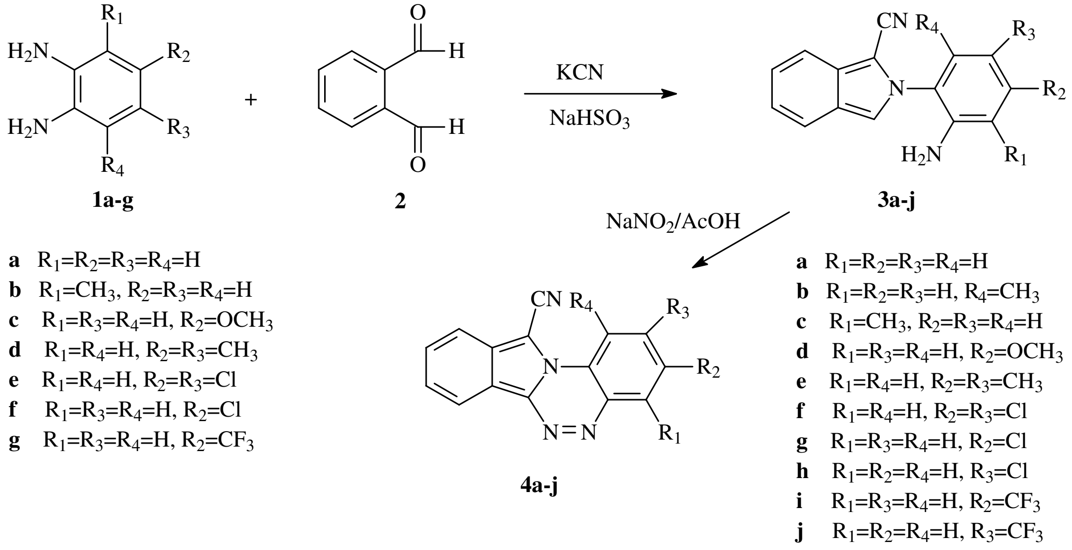
2-(2-Aminoaryl)-1-cyanoisoindoles **3a–j** appeared valuable and versatile intermediates for the synthesis of the new ring system isoindolo[2,1-*c*]benzo[1,2,4]triazines **4a–j**.

Table 1. Calculated free energies of binding obtained by AutoDock

Compound	ΔG (kcal/mol)
4a	−22.77
4b	−23.88
4c	−23.10
4d	−23.72
4e	−23.25
4f	−19.96
4g	−21.74
4h	−21.21
4i	−21.75
4j	−24.47
Actinomycin D	−10.36

Derivatives **3a–j** were prepared in good yield (40–95%) by a Strecker type synthesis between substituted *o*-phenylendiamines **1a–g** and phthaloyldicarboxaldehyde **2** in the presence of potassium cyanide and sodium hydrogensulfite. In the case of the monosubstituted *o*-phenylendiamines **1b**, **1f**, and **1g**, depending on the amino group involved in the cyclization to the isoindole nucleus two isomers were isolated. In particular from **1b** were isolated **3b** and **3c**; from **1f** were isolated **3g** and **3h**; from **1g** were isolated **3i** and **3j**. Instead in the case of **1c** (4-methoxy substituted) compound **3d** only was isolated due to the enhanced reactivity of the amino group placed in para position with respect to the methoxy substituent. Diazotization of derivatives **3a–j** with a stoichiometric amount of sodium nitrite at room temperature in acetic acid and successive cyclization of the resulting diazonium group by an intramolecular coupling with the position 3 of the isoindole afforded the title ring system in excellent yields (84–98%).

The structure of all compounds synthesized was confirmed by their analytical and spectral data (IR, ^{1}H, and ^{13}C NMR).



1a-g **2** **3a-j** **4a-j**

1a-g:

a $R_1=R_2=R_3=R_4=H$
b $R_1=CH_3$, $R_2=R_3=R_4=H$
c $R_1=R_3=R_4=H$, $R_2=OCH_3$
d $R_1=R_4=H$, $R_2=R_3=CH_3$
e $R_1=R_4=H$, $R_2=R_3=Cl$
f $R_1=R_3=R_4=H$, $R_2=Cl$
g $R_1=R_3=R_4=H$, $R_2=CF_3$

3a-j, **4a-j**:

a $R_1=R_2=R_3=R_4=H$
b $R_1=R_2=R_3=H$, $R_4=CH_3$
c $R_1=CH_3$, $R_2=R_3=R_4=H$
d $R_1=R_3=R_4=H$, $R_2=OCH_3$
e $R_1=R_4=H$, $R_2=R_3=CH_3$
f $R_1=R_4=H$, $R_2=R_3=Cl$
g $R_1=R_3=R_4=H$, $R_2=Cl$
h $R_1=R_2=R_4=H$, $R_3=Cl$
i $R_1=R_3=R_4=H$, $R_2=CF_3$
j $R_1=R_2=R_4=H$, $R_3=CF_3$

2.3. Biology

All the synthesized derivatives have been evaluated, by the National Cancer Institute (NCI, Bethesda, USA), in the 3-cell line (MCF7-breast, NCI-H460-non-small cell lung, SF-268-CNS), one dose primary anti-cancer assay (Table 2). A compound is considered active when it reduces the growth of any of the cell lines to 32% or less (negative numbers indicate cell kill).

The compounds, which passed the criteria set by the NCI for activity in this assay, were scheduled automatically for evaluation against the full panel of tumor cell lines.[16] Four isoindolo-benzo-triazine derivatives of type **4** were selected for evaluation against the full NCI panel of about 50 human cancer cell lines.

This panel is organized into subpanels derived from nine different human cancer types: leukemia, melanoma, lung, colon, renal, ovarian, breast, prostate, and CNS. The compounds were tested at five concentrations at 10-fold dilution the highest being 10^{-4} M and the others 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} M. The following parameters were determined for every cell line: GI_{50} (concentration inhibiting 50% net cell growth), TGI (concentration that totally inhibited net cell growth), and LC_{50} (concentration leading to 50% net cell death). For each of these parameters the average values of mean graph midpoint (MG_MID) were calculated.[17]

An evaluation of the data reported in Table 3 pointed out that compounds **4a**, **f**, **i**, and **j** exhibited antineoplastic activity against most of the human cell lines. The most active compounds are **4a** and**4j** in terms either of pGI_{50} (mean value 4.47 and 4.46, respectively) and percentage of sensitive cell lines out of the total number of cell lines investigated (83% and 89%, respectively).

Isoindolo-benzo-triazines **4a**, **f**, **i**, and **j** showed inhibitory effects in the growth of a wide range of cancer cell lines and most of susceptible tumors were generally inhibited at micromolar concentration (see Supporting information). Compound **4j**, bearing a trifluoromethyl group, was shown to be selective with respect to the leukemia subpanel having all the subpanel cell lines pGI_{50} in the range 4.15–6.67. The most sensitive cell lines are MOLT-4 ($pGI_{50} = 4.98$) and SR ($pGI_{50} = 6.67$). Excellent response was obtained in the nonsmall lung cancer; in fact the calculated pGI_{50} MG_MID values for the subpanel related to the active compounds are always higher than that of the over all cell lines (ΔMG_MID = 0.51) The most sensitive cell lines resulted in EKVX and NCI-522 ($pGI_{50} \geqslant 8.00$ and 4.77, respectively). Derivative **4a** was selectively active with respect to the nonsmall lung cancer and especially active against EKVX and A549/ATCC ($pGI_{50} = 4.90$ and 4.87, respectively). It also showed selectivity with respect to COLO-205 ($pGI_{50} = 5.03$) of colon cancer, LOX IMVI and M14 ($pGI_{50} = 5.19$ and 4.83, respectively) of melanoma subpanel, OVCAR-8 ($pGI_{50} = 4.95$) of ovarian cancer, and MCF7 ($pGI_{50} = 4.85$), BT-549 ($pGI_{50} = 4.92$) of breast cancer. Derivative **4i** had low pGI_{50} mean value ($pGI_{50} = 4.90$) but showed a selectivity concerning the breast cancer MCF7 cell line ($pGI_{50} = 4.97$).

Table 2. One dose primary anti-cancer assay for compounds **4**[a]

Compound	Breast MCF7	Non-small cell-lung NCI-H460	CNS SF-268	Compound selected
4a	26	21	95	Y
4b	69	50	88	N
4c	72	118	105	N
4d	63	86	104	N
4e	76	48	76	N
4f	80	17	68	Y
4g	109	109	114	N
4h	51	61	86	N
4i	65	6	82	Y
4j	83	8	96	Y

[a] Compounds were tested at the concentration of 10–4 M; exposure time 48 h.

Table 3. Overview of the results of the in vitro antitumor screening for compounds **4a**, **f**, **i**, **j**[a]

Compound	N[b]	pGI_{50}[c]		
		n[d]	Range	MG_MID[e]
4a	42	35	5.19–4.00	4.47
4f	53	42	4.70–4.00	4.31
4i	42	32	4.97–4.00	4.27
4j	53	47	>8.00–4.00	4.46

[a] Data obtained from NCI's in vitro disease-oriented human tumour cells screen.

[b] N is the number of cell lines investigated.

[c] pGI_{50} is the −log of the molar concentration that inhibits 50% net cell growth.

[d] n is the number of cell lines giving positive pGI_{50}.

[e] MG_MID = mean graph midpoint = arithmetical mean value for all tested cancer cell lines. If the identical effect was not attainable within the used concentrated interval, the highest tested concentration was used for the calculation.

In order to discern the mechanism of action of isoindolo-benzo-triazines we performed COMPARE computations[18] for derivatives **4a**, **f**, **i**, **j** against the NCI 'Standard Agents' database. All compounds had a Pearson correlation coefficient (PCC) <0.6 suggesting that the antiproliferative activity of isoindolo[2,1-*c*]benzo[1,2,4] triazines would be mechanistically unrelated to that of any known drug. We also utilized the facility 3D-MIND (Drug Discovery and Data Mining Information for New Directions) projecting our compounds **4a**, **f**, **i**, **j** onto the 'Complete DTP Map' a self-organizing map (SOM) based on cytotoxicity measurements from the NCI anti-cancer drug-screening database (http://spheroid.ncifcrf.gov) which cluster these data in the high-dimensional GI_{50} space and provide a means of its visual translation into a two dimensional anti-cancer map. The Complete map is divided in six regions designated alphabetically (M, mitosis; N, membrane transport and integrity; P, phosphatase- and kinase cell cycle regulation; S, nucleic acid synthesis; Q, R, arbitrarily regions) which share the same pattern of growth inhibition, and which substantially reflect their molecular targets and modes of action.[19] The results of this analysis on projected isoindolo-benzo-triazines indicate their location in the

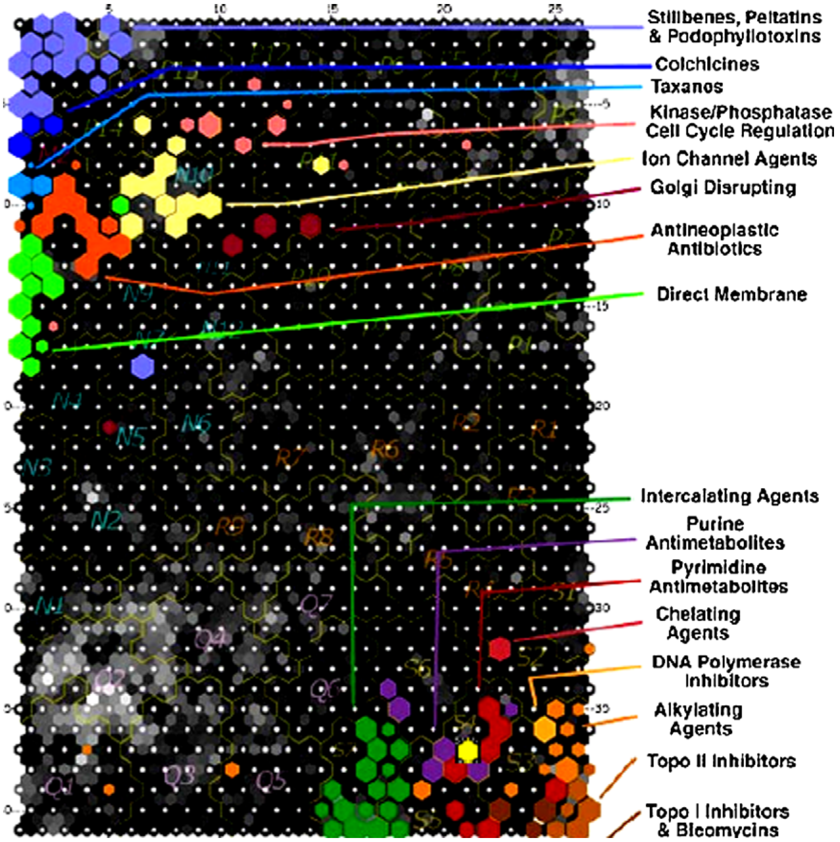


Figure 1. Projection of compound **4j** onto complete compound map (molecular activity classes).

S region map where antimetabolites of purine and pyrimidine nucleotides are found (Fig. 1).

It is our intention to undertake studies directed to elucidate the biochemical mechanism of action of this series of isoindolo[2,1-*c*]benzo[1,2,4]triazines.

3. Experimental

All melting points were taken on a Büchi-Tottoli capillary apparatus and are uncorrected; IR spectra were determined in bromoform with a Jasco FT/IR 5300 spectrophotometer; ^{1}H and ^{13}C NMR spectra were measured at 200 and 50.3 MHz, respectively, in DMSO-d_6 or CDCl$_3$ solution, using a Bruker AC series 200 MHz spectrometer (TMS as internal reference). Column chromatography was performed with Merck silica gel 230–400 Mesh ASTM or with Sepacore Buchi apparatus. Elemental analyses (C, H, and N) were within ±0.4% of the theoretical values.

3.1. Synthesis of substituted 1-cyano-2-(2′-aminophenyl) isoindoles (3a–j)

Method A. To a solution of 1.56 g (0.015 mol) of sodium hydrogen sulfite in water (38 ml) was added phthaloyldicarboxaldehyde **2** (2 g, 0.015 mol). The mixture was stirred until the solid was dissolved and the appropriate *o*-phenylenediamine **1a–d**, **g** (0.015 mol) was added, and the reaction mixture was heated on a steam bath for 30 min. Then KCN (3.39 g, 0.052 mol) in water (8.0 ml) was added and the mixture heated for an additional 90 min. The mixture was cooled and the solid formed was filtered and purified by chromatography.

Method B. Differs from Method A in that: (i) the *o*-phenylendiamine **1e**, **f** were added after dissolution in DMF (15 ml); (ii) after the addition of KCN the mixture was heated for 2 h; (iii) the mixture was poured into 200 ml of water and the solid formed was purified by chromatography.

3.1.1. 1-Cyano-2-(2′-aminophenyl)isoindole (3a). *Method A*. This compound was purified by flash chromatography using dichloromethane as eluent to give a solid: yield 65%; mp 150–151 °C. IR 3473, 3363 (NH$_2$), 2202 (CN) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 5.19 (2H, s, NH$_2$), 6.70 (1H, t, $J = 7.2$ Hz, H-5′), 6.94 (1H, d, $J = 7.2$ Hz, H-3′), 7.15–7.19 (3H, m, H-4′, H-5 and H-6′), 7.31 (1H, t, $J = 8.2$ Hz, H-6), 7.66 (1H, d, $J = 8.2$ Hz, H-4), 7.78 (1H, d, $J = 8.2$ Hz, H-7), 7.86 (1H, s, H-3). ^{13}C NMR (DMSO-d_6) δ 93.9 (s, C-1), 114.0 (s, CN), 115.8 (d, C-3′), 116.1 (d, C-5′), 117.5 (d, C-6′), 121.5 (d, C-4′), 122.2 (d, C-3), 122.4 (d, C-7), 122.5 (s, C-3a), 124.1 (s, C-1′), 125.5 (d, C-4), 127.8 (d, C-5), 130.4 (d, C-6), 131.2 (s, C-7a), 144.1 (s, C-2′). Anal. Calcd for $C_{15}H_{11}N_3$ (MW 233.27): C, 77.23; H, 4.75; N, 18.01%. Found: C, 77.46; H, 4.67; N, 18.12%.

3.1.2. 1-Cyano-2-(2′-amino-6′-methylphenyl)isoindole (3b). *Method A*. This compound was purified by Sepacore Buchi apparatus using dichloromethane/cyclohexane (7:3) as eluent to give a solid: yield 45%; mp 87–88 °C.

IR 3483, 3390 (NH_2), 2202 (CN) cm^{-1}. ^{1}H NMR ($CDCl_3$) *δ* 1.98 (3H, s, CH_3), 3.45 (2H, s, NH_2), 6.69 (1H, d, *J* = 8.1 Hz, H-3′), 6.75 (1H, d, *J* = 8.1 Hz, H-5′), 7.16 (1H, t, *J* = 8.1 Hz, H-4′), 7.20 (1H, t, *J* = 7.6 Hz, H-5), 7.26 (1H, t, *J* = 7.6 Hz, H-6), 7.35 (1H, s, H-3), 7.71 (1H, d, *J* = 7.6 Hz, H-4), 7.74 (1H, d, *J* = 7.6 Hz, H-7). ^{13}C NMR ($CDCl_3$) *δ* 17.0 (q, CH_3), 94.7 (s, C-1), 113.6 (s, CN), 114.1 (d, C-5′), 118.5 (d, C-3), 120.0 (d, C-7), 120.3 (d, C-4), 121.0 (d, C-3′), 123.2 (d, C-4′), 123.6 (s, C-6′), 124.8 (s, C-3a), 125.9 (d, C-5), 130.3 (s, C-1′), 130.6 (d, C-6), 136.3 (s, C-7a), 143.0 (s, C-2′). Anal. Calcd for $C_{16}H_{13}N_3$ (MW 247.30): C, 77.71; H, 5.30; N, 16.99%. Found: C, 77.45; H, 5.60; N, 17.08%.

3.1.3. 1-Cyano-2-(2′-amino-3′-methylphenyl)isoindole (3c). *Method A*. This compound was purified by Sepacore Buchi apparatus using dichloromethane/cyclohexane (7:3) as eluent to give a solid: yield 40%; mp 62–63 °C. IR 3481, 3388 (NH_2), 2200 (CN) cm^{-1}. ^{1}H NMR ($CDCl_3$) *δ* 2.25 (3H, s, CH_3), 3.55 (2H, s, NH_2), 6.80 (1H, t, *J* = 7.6 Hz, H-5′), 7.10 (1H, d, *J* = 7.6 Hz, H-6′), 7.14 (1H, d, *J* = 7.6 Hz, H-4′), 7.23 (1H, t, *J* = 8.1 Hz, H-5), 7.31 (1H, t, *J* = 8.1 Hz, H-6), 7.44 (1H, s, H-3), 7.69 (1H, d, *J* = 8.1 Hz, H-4), 7.73 (1H, d, *J* = 8.1 Hz, H-7). ^{13}C NMR ($CDCl_3$) *δ* 17.6 (q, CH_3), 92.2 (s, C-1), 113.0 (s, CN), 117.9 (d, C-5′), 118.3 (d, C-6′), 120.5 (d, C-3), 120.9 (d, C-7), 123.2 (d, C-4), 123.8 (s, C-3a), 124.1 (s, C-1′), 124.6 (s, C-3′), 125.5 (d, C-4′), 125.9 (d, C-5), 131.8 (s, C-7a), 131.9 (d, C-6), 140.5 (s, C-2′). Anal. Calcd for $C_{16}H_{13}N_3$ (MW 247.30): C, 77.71; H, 5.30; N, 16.99. Found: C, 77.59; H, 5.45; N, 16.87.

3.1.4. 1-Cyano-2-(2′-amino-4′-methoxyphenyl)isoindole (3d). *Method A*. This compound was purified by flash chromatography using dichloromethane as eluent to give a solid: yield 95%; mp 116–117 °C. IR 3465, 3375 (NH_2), 2198 (CN) cm^{-1}. ^{1}H NMR ($CDCl_3$) *δ* 3.64 (2H, br s, NH_2), 3.80 (3H, s, CH_3), 6.38 (1H, br s, H-3′), 6.42 (1H, d, *J* = 7.9 Hz, H-5′), 7.12 (1H, d, *J* = 7.9 Hz, H-6′), 7.14 (1H, t, *J* = 8.6 Hz, H-5), 7.29 (1H, t, *J* = 8.6 Hz, H-6), 7.41 (1H, s, H-3), 7.66 (1H, d, *J* = 8.6 Hz, H-4), 7.70 (1H, d, *J* = 8.6 Hz, H-7). ^{13}C NMR ($CDCl_3$) *δ* 55.4 (q, CH_3), 95.4 (s, C-1), 101.4 (d, C-3′), 104.5 (d, C-5′), 113.9 (s, CN), 117.5 (s, C-1′), 118.2 (d, C-6′), 120.8 (d × 2, C-3 e C-7), 123.1 (d, C-4), 124.5 (s, C-3a), 125.8 (d, C-5), 128.7 (d, C-6), 131.7 (s, C-7a), 143.3 (s, C-2′), 161.5 (s, C-4′). Anal. Calcd for $C_{16}H_{13}N_3O$ (MW 263.30): C, 72.99; H, 4.98; N, 15.96%. Found: C, 71.89; H, 5.03; N, 16.22%.

3.1.5. 1-Cyano-2-(2′-amino-4′,5′-dimethylphenyl)isoindole (3e). *Method A*. This compound was purified by flash chromatography using dichloromethane as eluent to give a solid: yield 85%; mp 119–120 °C. IR 3465, 3375 (NH_2), 2202 (CN) cm^{-1}. ^{1}H NMR ($CDCl_3$) *δ* 2.17 (3H, s, CH_3), 2.22 (3H, s, CH_3), 3.44 (2H, s, NH_2), 6.64 (1H, s, H-3′), 6.95 (1H, s, H-6′), 7.12 (1H, t, *J* = 7.6 Hz, H-5), 7.27 (1H, t, *J* = 7.6 Hz, H-6), 7.38 (1H, s, H-3), 7.64 (1H, d, *J* = 7.6 Hz, H-4), 7.68 (1H, d, *J* = 7.6 Hz, H-7). ^{13}C NMR ($CDCl_3$) *δ* 18.6 (q, CH_3), 19.7 (q, CH_3), 95.1 (s, C-1), 114.0 (s, CN), 118.1 (d, C-3′), 118.2 (d, C-6′), 120.6 (d, C-3), 120.9 (d, C-7), 121.8 (s, C-3a), 123.1 (d, C-4), 124.5 (s, C-1′), 125.8 (d, C-5), 127.0 (s, C-5′), 128.2 (d, C-6), 131.7 (s, C-7a), 139.6 (s, C-4′), 139.7 (s, C-2′). Anal. Calcd for $C_{17}H_{15}N_3$ (MW 261.33): C, 78.13; H, 5.79; N, 16.08%. Found: C, 78.34; H, 5.67; N, 15.89%.

3.1.6. 1-Cyano-2-(2′-amino-4′,5′-dichlorophenyl)isoindole (3f). *Method B*. This compound was purified by flash chromatography using dichloromethane as eluent to give a solid: yield 77%; mp 71–72 °C. IR 3394, 3340 (NH_2), 2200 (CN) cm^{-1}. ^{1}H NMR (DMSO-d_6) *δ* 5.75 (2H, s, NH_2), 7.12 (1H, s, H-3′), 7.16 (1H, t, *J* = 8.4 Hz, H-5), 7.32 (1H, t, *J* = 8.4 Hz, H-6), 7.58 (1H, s, H-6′), 7.66 (1H, d, *J* = 8.4 Hz, H-4), 7.78 (1H, d, *J* = 8.4 Hz, H-7), 7.91 (1H, s, H-3). ^{13}C NMR (DMSO-d_6) *δ* 94.0 (s, C-1), 113.8 (s, CN), 115.8 (s, C-1′), 116.2 (d, C-3′), 117.5 (d, C-6′), 121.6 (d, C-3), 121.7 (s, C-3a), 122.4 (d, C-7), 122.6 (d, C-4), 124.2 (s, C-5′), 125.8 (d, C-5), 129.6 (d, C-6), 131.2 (s, C-7a), 132.9 (s, C-4′), 144.7 (s, C-2′). Anal. Calcd for $C_{15}H_9Cl_2N_3$ (MW 302.16): C, 59.63; H, 3.00; N, 13.91%. Found: C, 59.72; H, 3.23; N, 13.81%.

3.1.7. 1-Cyano-2-(2′-amino-4′-chlorophenyl)isoindole (3g). *Method B*. This compound was purified by flash chromatography using dichloromethane as eluent to give a solid: yield 40%; mp 170–171 °C. IR 3475, 3390 (NH_2), 2202 (CN) cm^{-1}. ^{1}H NMR (DMSO-d_6) *δ* 5.53 (2H, s, NH_2), 6.70 (1H, d, *J* = 8.4 Hz, H-5′), 6.93 (1H, s, H-3′), 7.15 (1H, t, *J* = 7.9 Hz, H-5), 7.22 (1H, d, *J* = 7.9 Hz, H-4), 7.31 (1H, t, *J* = 7.9 Hz, H-6), 7.65 (1H, d, *J* = 8.4 Hz, H-6′), 7.79 (1H, d, *J* = 7.9 Hz, H-7), 7.86 (1H, s, H-3). ^{13}C NMR (DMSO-d_6) *δ* 94.0 (s, C-1), 113.9 (s, CN), 115.0 (d, C-3′), 115.2 (d, C-5′), 117.6 (d, C-3), 121.2 (s, C-1′), 121.6 (d, C-7), 122.3 (d, C-4), 122.6 (d, C-6′), 124.2 (s, C-3a), 125.7 (d, C-5), 129.7 (d, C-6), 131.3 (s, C-4′), 134.9 (s, C-7a), 145.8 (s, C-2′). Anal. Calcd for $C_{15}H_{10}ClN_3$ (MW 267.72): C, 67.30; H, 3.76; N, 15.70%. Found: C, 67.49; H, 3.55; N, 15.83%.

3.1.8. 1-Cyano-2-(2′-amino-5′-chlorophenyl)isoindole (3h). *Method B*. This compound was purified by flash chromatography using dichloromethane as eluent to give a solid: yield 55%; mp 131–132 °C. IR 3471, 3365 (NH_2), 2200 (CN) cm^{-1}. ^{1}H NMR (DMSO-d_6) *δ* 5.41 (2H, s, NH_2), 6.94 (1H, d, *J* = 8.6 Hz, H-4′), 7.17 (1H, t, *J* = 7.6 Hz, H-5) 7.20–7.36 (3H, m, H-4, H-6, H-6′), 7.67 (1H, d, *J* = 8.6 Hz, H-3′), 7.79 (1H, d, *J* = 7.6 Hz, H-7), 7.90 (1H, s, C-3). ^{13}C NMR (DMSO-d_6) *δ* 95.3 (s, C-1), 114.0 (s, CN), 117.3 (d, C-3′), 117.6 (d, C-3), 118.4 (s, C-3a), 121.6 (d, C-7), 122.3 (d, C-4), 122.6 (d, C-5), 122.8 (s, C-5′), 124.2 (s, C-1′), 125.8 (d, C-4′), 127.5 (d, C-6), 130.4 (d, C-6′), 131.3 (s, C-7a), 145.5 (s, C-2′). Anal. Calcd for $C_{15}H_{10}ClN_3$ (MW 267.72): C, 67.30; H, 3.76; N, 15.70%. Found: C, 67.12; H, 3.81; N, 15.63%.

3.1.9. 1-Cyano-2-(2′-amino-4′-trifluoromethylphenyl)isoindole (3i). *Method A*. This compound was purified by Sepacore Buchi apparatus using cyclohexane/ethyl-acetate (9:1) as eluent to give an oil: yield 50%. IR: 3481, 3371 (NH_2), 2202 (CN) cm^{-1}. ^{1}H NMR (DMSO-d_6) *δ* 5.89 (2H, s, NH_2), 7.09(1H, t, *J* = 8.0 Hz, H-5), 7.21 (1H, s, H-3′) 7.26 (1H, d, *J* = 7.8 Hz, H-5′), 7.39 (1H, d, $J = 7.8$ Hz, H-6′), 7.45 (1H, t, $J = 8.0$ Hz, H-6) 7.49 (1H, d, $J = 8.0$ Hz, H-4), 7.69 (1H, d, $J = 8.0$ Hz, H-7), 7.89 (1H, s, H-3). ^{13}C NMR (DMSO-d_6) δ 93.8 (s, C-1), 109.2 (d, C-5′), 114.0 (s, CN), 115.6 (s, CF_3), 116.4 (d, C-3), 117.8 (d, C-3′), 119.0 (d, C-7), 121.2 (s, C-3a), 123.0 (d, C-4), 124.7 (d, C-6′), 126.0 (d, C-5), 126.2 (d, C-6), 126.8 (s, C-4′), 127.2 (s, C-1′), 132.0 (s, C-7a), 144.8 (s, C-2′). Anal. Calcd for $C_{16}H_{10}F_3N_3$ (MW 301.27): C, 63.79; H, 3.35; N, 18.92%. Found: C, 63.58; H, 3.18; N, 19.03%.

3.1.10. 1-Cyano-2-(2′-amino-5′-trifluoromethylphenyl)-isoindole (3j).

Method A. This compound was purified by Sepacore Buchi apparatus using cyclohexane/ethylacetate (9:1) as eluent to give an oil: yield 44%. IR 3481, 3381 (NH_2), 2200 (CN) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 5.99 (2H, s, NH_2), 7.02 (1H, d, $J = 8.9$ Hz H-3′), 7.17 (1H, t, $J = 8.0$ Hz, H-5), 7.33 (1H, t, $J = 8.0$ Hz, H-6), 7.57 (1H, s, H-6′), 7.58 (1H, d, $J = 8.0$ Hz, H-4), 7.67 (1H, d, $J = 8.9$ Hz, H-4′), 7.79 (1H, d, $J = 8.0$ Hz, H-7), 7.94 (1H, s, H-3). ^{13}C NMR (DMSO-d_6) δ 94.0 (s, C-1), 113.9 (s, CN), 114.9 (s, C-5′), 115.5 (s, CF_3), 115.9 (d, C-3), 117.6 (d, C-7), 120.2 (s, C-3a), 121.3 (d, C-3′), 122.5 (d, C-4), 124.2 (s, C-1′), 125.6 (d, C-5), 125.7 (d, C-6), 127.5 (d, C-4′), 127.6 131.3 (s, C-7a), 147.8 (s, C-2′). Anal. Calcd for $C_{16}H_{10}F_3N_3$ (MW 301.27): C, 63.79; H, 3.35; N, 18.92%. Found: C, 63.84; H, 3.21; N, 18.87%.

3.2. General method for the synthesis of isoindolo[2,1-*c*]benzo[1,2,4]triazines (4a–j)

To a solution of appropriate 1-cyano-2-(2′-aminophenyl)-isoindole **3a–j** (3 mmol) in 5 ml of acetic acid, a solution of sodium nitrite 3 mmol (210 mg) in water (1 ml) was added. The mixture was stirred at room temperature for 24 h then poured onto crushed ice. The precipitate was filtered, air dried, and purified by flash chromatography with dichloromethane as eluent to give:

3.2.1. 11-Cyano-isoindolo[2,1-*c*]benzo[1,2,4]triazine (4a).

Yield 84%; mp 266–267 °C. IR 2200 (CN) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.66 (1H, t, $J = 7.4$ Hz, H-8), 7.76 (1H, t, $J = 7.4$ Hz, H-9), 7.89 (1H, t, $J = 8.0$ Hz, H-2), 7.93 (1H, t, $J = 8.0$ Hz, H-3), 7.98 (1H, d, $J = 7.4$ Hz, H-7), 8.67 (1H, d, $J = 8.0$ Hz, H-1), 8.69 (1H, d, $J = 7.4$ Hz, H-10), 9.11 (1H, d, $J = 8.0$ Hz, H-4). ^{13}C NMR ($CDCl_3$) δ 88.6 (s), 114.9 (s), 115.4 (d), 117.4 (d), 120.2 (s), 120.7 (d), 124.4 (s), 126.4 (d), 128.8 (d), 129.7 (d), 131.2 (d), 132.4 (s), 132.9 (d), 134.6 (s), 139.8 (s). Anal. Calcd for $C_{15}H_8N_4$ (MW 244.26): C, 73.76; H, 3.30; N, 22.94%. Found: C, 73.67; H, 3.47; N, 23.04%.

3.2.2. 11-Cyano-1-methylisoindolo[2,1-*c*]benzo[1,2,4]triazine (4b).

Yield 90%; mp 217–218 °C. IR 2201 (CN) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 3.14 (3H, s, CH_3), 7.68 (1H, t, $J = 8.0$ Hz, H-8), 7.72–7.87 (3H, m, H-9, H-3 and H-2), 8.03 (1H, d, $J = 8.0$ Hz, H-7), 8.49 (1H, dd, $J = 6.9$, 2.4 Hz, H-4), 8.73 (1H, d, $J = 8.0$ Hz, H-10). ^{13}C NMR ($CDCl_3$) δ 23.2 (q), 94.1 (s), 115.3 (s), 117.7 (d), 120.0 (s), 120.7 (d), 124.5 (s), 126.7 (d), 128.3 (d), 128.4 (d), 129.7 (d), 130.3 (s), 133.8 (s), 135.6 (d), 137.0 (s), 141.8 (s). Anal. Calcd for $C_{16}H_{10}N_4$ (MW 258.28): C, 74.41; H, 3.90; N, 21.69%. Found: C, 74.35; H, 4.03; N, 21.72%.

3.2.3. 11-Cyano-4-methylisoindolo[2,1-*c*]benzo[1,2,4]triazine (4c).

Yield 98%; mp 255–256 °C. IR 2198 (CN) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.37 (3H, s, CH_3), 7.71 (1H, t, $J = 8.4$ Hz, H-8), 7.74–7.85 (2H, m, H-9 and H-3), 7.89 (1H, t, $J = 7.8$ Hz, H-2), 8.05 (1H, d, $J = 7.8$ Hz, H-1), 8.78 (1H, d, $J = 8.4$ Hz, H-7), 9.03 (1H, d, $J = 8.4$ Hz, H-10). ^{13}C NMR ($CDCl_3$) δ 18.3 (q), 88.3 (s), 113.1 (d), 115.2 (s), 117.4 (d), 120.0 (s), 120.8 (d), 124.5 (s), 126.2 (d), 129.7 (d), 129.8 (d), 131.8 (s), 132.7 (d), 134.7 (s), 138.7 (s), 141.0 (s). Anal. Calcd for $C_{16}H_{10}N_4$ (MW 258.28): C, 74.41; H, 3.90; N, 21.69%. Found: C, 74.29; H, 4.00; N, 21.81%.

3.2.4. 11-Cyano-3-methoxyisoindolo[2,1-*c*]benzo[1,2,4]triazine (4d).

Yield 87%; mp 256–257 °C. IR 2198 (CN) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 4.08 (3H, s, CH_3), 7.60–7.66 (2H, m, H-4 and H-10), 7.77 (1H, t, $J = 8.0$ Hz, H-8), 7.78 (1H, t, $J = 8.0$ Hz, H-9), 7.98 (1H, d, $J = 7.0$ Hz, H-2), 8.67 (1H, d, $J = 8.0$ Hz, H-7), 9.01 (1H, d, $J = 7.0$ Hz, H-1). ^{13}C NMR (DMSO-d_6) δ 56.1 (q), 93.3 (s), 109.8 (d), 111.5 (s), 116.5 (d), 117.2 (d), 119.9 (s), 120.4 (d), 123.9(d), 126.0 (d), 126.6 (s), 129.5 (d), 130.6 (s), 132.0 (s), 135.9 (s), 145.5 (s). Anal. Calcd for $C_{16}H_{10}N_4O$ (MW 274.28): C, 70.07; H, 3.67; N, 20.43%. Found: C, 69.91; H, 3.74; N, 20.57%.

3.2.5. 11-Cyano-2,3-dimethylisoindolo[2,1-*c*]benzo[1,2,4]-triazine (4e).

Yield 96%; mp 269–270 °C. IR 2195 (CN) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.57 (3H, s, CH_3), 2.63 (3H, s, CH_3), 7.64 (1H, t, $J = 7.8$ Hz, H-8), 7.75 (1H, t, $J = 7.8$ Hz, H-9), 8.00 (1H, d, $J = 7.8$ Hz, H-7), 8.40 (1H, s, H-1), 8.72 (1H, d, $J = 7.8$ Hz, H-10), 8.87 (1H, s, H-4). ^{13}C NMR ($CDCl_3$) δ 20.1 (q), 21.3 (q), 87.6 (s), 115.0 (d), 115.3 (s), 117.4 (d), 120.0 (s), 120.8 (d), 122.8 (s), 125.9 (d), 129.5 (d), 130.6 (d), 132.6 (s), 134.7 (s), 139.0 (s), 139.1 (s), 144.6 (s). Anal. Calcd for $C_{17}H_{12}N_4$ (MW 272.31): C, 74.98; H, 4.44; N, 20.57%. Found: C, 74.85; H, 4.61; N, 20.40%.

3.2.6. 11-Cyano-2,3-dichloroisoindolo[2,1-*c*]benzo[1,2,4]-triazine (4f).

Yield 87%; mp 241–242 °C. IR 2200 (CN) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.73 (1H, t, $J = 8.0$ Hz, H-8), 7.82 (1H, t, $J = 8.0$ Hz, H-9), 8.54 (1H, s, H-1), 8.74 (1H, d, $J = 8.0$ Hz, H-7), 8.76 (1H, s, H-4); 9.38 (1H, d, $J = 8.0$ Hz, H-10). ^{13}C NMR ($CDCl_3$) δ 88.9 (s), 114.9 (s), 117.0 (s), 117.7 (s), 118.8 (s), 120.9 (d), 125.2 (d), 127.3 (d), 129.7 (s), 130.5 (d), 131.8 (d), 132.2 (d), 132.7 (s), 134.2 (s), 163.6 (s). Anal. Calcd for $C_{15}H_6Cl_2N_4$ (MW 313.15): C, 57.53; H, 1.93; N, 22.64%. Found: C, 57.36; H, 2.05;N, 22.75%.

3.2.7. 3-Chloro-11-cyanoisoindolo[2,1-*c*]benzo[1,2,4]triazine (4g).

Yield 85%; mp 267–268 °C. IR 2200 (CN) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 7.78 (1H, t, $J = 7.6$ Hz, H-8), 7.89 (1H, t, $J = 7.6$ Hz, H-9), 8.11 (1H, d, $J = 8.4$ Hz, H-2), 8.24 (1H, d, $J = 7.6$ Hz, H-7), 8.71 (1H, d, $J = 8.4$ Hz, H-1), 8.79 (1H, s, H-4), 9.00 (1H, d, J = 7.6 Hz, H-10). ^{13}C NMR (DMSO-d_6) δ 92.7 (s), 114.7 (s), 117.3 (d), 117.6 (d), 119.5 (s), 120.3 (d), 123.4 (s), 126.6 (d), 129.0 (d), 129.9 (d), 131.8 (s), 133.1 (d), 140.0 (s), 148.1 (s), 158.9 (s). Anal. Calcd for $C_{15}H_7ClN_4$ (MW 278.70): C, 64.64; H, 2.53; N, 20.10%. Found: C, 64.49; H, 2.77; N, 19.99%.

3.2.8. 2-Chloro-11-cyanoisoindolo[2,1-*c*]benzo[1,2,4]triazine (4h). Yield 84%; mp 222–223 °C. IR 2200 (CN) cm^{-1}. ^{1}H NMR (CDCl$_3$) δ 7.68–8.01 (4H, m, H-3, H-4, H-8 and H-9), 8.61 (1H, s, H-1), 8.71 (1H, d, J = 7.4 Hz, H-7), 9.05 (1H, d, J = 7.4 Hz, H-10). ^{13}C NMR (CDCl$_3$) δ 92.8 (s), 115.2 (s), 120.8 (d × 2), 122.9 (s), 125.6 (d), 128.2 (s), 130.2 (d × 2), 131.6 (s), 132.5 (d), 133.0 (d), 134.9 (s), 137.0 (s), 153.7 (s). Anal. Calcd for $C_{15}H_7ClN_4$ (MW 278.70): C, 64.64; H, 2.53; N, 20.10%. Found: C, 64.70; H, 2.33; N, 20.02%.

3.2.9. 11-Cyano-3-trifluoromethylisoindolo[2,1-*c*]benzo-[1,2,4] triazine (4i). Yield 95%; mp 170–171 °C. IR 2200 (CN) cm^{-1}. ^{1}H NMR (CDCl$_3$) δ 7.70 (1H, t, J = 8.3 Hz, H-8), 7.80 (1H, t, J = 8.3 Hz, H-9), 8.01 (1H, d, J = 8.0 Hz, H-1), 8.60 (1H, d, J = 8.3 Hz, H-10), 8.74 (1H, d, J = 8.3 Hz, H-7), 8.77 (1H, d, J = 8.0 Hz, H-2), 9.38 (1H, s, H-4). ^{13}C NMR (CDCl$_3$) δ 90.0 (s), 113.5 (d), 114.2 (s), 115.4 (s), 116.6 (s), 117.7 (d), 120.7 (d), 124.1 (s), 125.1 (d), 125.5 (s), 127.3 (d), 130.3 (d), 131.2 (s), 132.2 (d), 134.6 (s), 140.32 (s). Anal. Calcd for $C_{16}H_7F_3N_4$ (MW 312.25): C, 61.54; H, 2.26; N, 17.94%. Found: C, 61.49; H, 2.41; N, 17.81%.

3.2.10. 11-Cyano-2-trifluoromethylisoindolo[2,1-*c*]benzo-[1,2,4]triazine (4j). Yield 86%; mp 135–137 °C. IR 2200 (CN) cm^{-1}. ^{1}H NMR (CDCl$_3$) δ 7.74 (1H, t, J = 8.0 Hz, H-8), 7.83 (1H, t, J = 8.0 Hz, H-9), 8.06 (1H, d, J = 8.0 Hz, H-7), 8.16 (1H, d, J = 9.0 Hz, H-3), 8.79 (1H, d, J = 8.0 Hz, H-10); 8.95 (1H, s, H-1), 9.27 (1H, d, J = 9.0 Hz, H-4). ^{13}C NMR (CDCl$_3$) δ 92.4 (s), 114.3 (s), 114.5 (s), 116.7 (d), 117.7 (d), 120.3 (s), 120.6 (s), 120.9 (d), 125.7 (s), 126.3 (s), 127.3 (d), 128.6 (d), 128.8 (d), 130.4 (d), 132.8 (s), 138.9 (s). Anal. Calcd for $C_{16}H_7F_3N_4$ (MW 312.25): C, 61.54; H, 2.26; N, 17.94%. Found: C, 61.37; H, 2.45; N, 18.04%.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.09.054.

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